Journal of Metabolic Syndrome

Refaat and Hamdoun, J Metabolic Synd 2014, 3:4
DOI: 10.4172/2167-0943.1000156

Research Article Open Access

Improvement of Conception in Sheep Using Different Hormonal Treatments during Mating and their Influence on the Antioxidant Status

Derar Refaat[1*] **and Hamdoun**[2]

[1]*Department of Theriogenology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt*

[2]*Department of Animal & Poultry Production, Faculty of Agriculture, Sohag University, Sohag, Egypt*

***Corresponding author:** Derar Refaat, Assiut University, Faculty of Veterinary Medicine, Department of Theriogenology, Assiut, Egypt, E-mail: derar40@gmail.com and derar40@yahoo.com

Received date: May 30, 2014, **Accepted date:** July 14, 2014, **Published date:** July 19, 2014

Abstract

The objective of this study was to compare the effects of GnRH, prostaglandin F2α (PGF2α) and oxytocin treatments at the time of natural mating on the conception rate (CR) of non lactating pluriparous ewes. All ewes (n=61) were served naturally by fertile rams every 12 hours after the beginning of estrus. After natural mating, ewes were randomly assigned into four treatment groups; G1 received PGF2α (n=14); G2 received GnRH (n=12); G3received oxytocin (n=15) and G4 or control received placebo (n=20). Pregnancy diagnosis was performed 25 days post-insemination by transrectal ultrasonography. Ewes were bled at the day of mating and every 10 days till Day 50 post mating to determine the changes in the total antioxidants during the first third of pregnancy. Pregnancy rate was higher ($P<0.05$) for all the treatment groups (69.33%) compared with the control group (55.54%). Litter size did not differ between groups except for oxytocin group. Ewe lambs dominate male in this study and the sex ratio unexpectedly preferred them. Total antioxidants did not differ significantly between groups in the present study but they were at their lowest values during estrus in all the studied groups. Gestation length, birth weight, number of services, body weight did not affect the pregnancy rate. It could be concluded that treatments with GnRH and PGF and oxytocin at the time of service could improve conception rate in pluriparous ewes.

Keywords: Antioxidants; Conception; Ewe; Hormones

Introduction

Many trials have been attempted to increase fertility in ewes. Gonadotropin-releasing hormone (GnRH) and its analogues administered at the time of artificial insemination (AI) are the most common treatments in management programmers for sheep flocks [1,2]. Improvement of the conception following GnRH treatment has been attributed to the prevention of an ovulation failure or a reduced variation in the interval between the onset of estrus and ovulation [3]. However, the results are controversial after GnRH treatment of lactating cows. Many previous works reported that conception rate in cows was improved [3], while others reported no effect on pregnancy rate was obtained [1,4]. Oxytocin and PGF2α have been shown as essential parts of ovulation process [5,6] and has been known that the increase of uterine and oviduct contractility (Hawk, 1983) affects the sperm transport. There are few studies focused on the effect of PGF2α administration at the time of AI on pregnancy [6]. Oxytocin was used to increase conception rate by improving the sperm transport in the female reproductive tract of several species [7-9]. Clitoral massage which probably releases oxytocin following artificial insemination increased pregnancy in beef cows [10]. The administration of oxytocin following AI also increased CR in lactating dairy cows [9] but in another study it had hardly any effect on pregnancy in cows [11]. The objective of the present study was to study the effect of different hormonal treatments used to improve the reproductive efficiency in ewes on different reproductive parameters and antioxidant profiles after natural mating in subtropics.

Materials and Methods

This work was carried out in the Animal Production Experimental Farm, Animal and Poultry Production Department, Faculty of Agriculture, Sohag University, Egypt (latitude 28°07´N and 30°33´E)

Animals and management

Sixty one ewes Sohagi healthy, pluriparous, non parturient and non lactating ewes were used in this study. Ewes were kept away from rams before the beginning of the study and housed in semi-open pens. Ewes were fed on a concentrate mixture with wheat straw and green fodder, providing 14% crude protein and 70% total digestible nutrients during the experimental period (from September 15th till December 31st). Water was available all time. Estrus was detected using well trained teasers and personnel. Estrous ewes were mated with fertile rams every 12 hours till the end of estrus. Immediately after the last mating, animals were assigned into four groups: G1 (n=14) received 15 mg of Dinoprost IM (PGF2α. Lutalyse, Pharmacia & Upjohn, NY); G2 (n=12) received 25 µg Gonadorelin IM (Factrel, Fort Dodge, IA, USA); G3 (n=15) treated with 20 IU oxytocin IM (Biomeda-MTC Animal Health Inc., Cambridge, Ontario, Canada) and G4 (control group, n=20) received 5 ml normal saline IM. Doses and route of administration of each drug in the present study were administered according to the instructions of the manufacturers. Pregnancy was diagnosed on Day 25 post mating for all animals using a real-time, B-mode echocamera (EUB-405B, Hitachi, Tokyo, Japan) attached with a 5-7.5 MHz transducer. Visualization of a fluid-filled uterine horn with embryonic vesicles and the presence of an embryo were used as positive indicators for pregnancy. Pregnancy rate was calculated as the number of ewes diagnosed pregnant divided by the number of mated ewes.

Serum total antioxidants status

Blood samples were collected from animals beginning on day 0 (day of treatment) and every 10 days till day 50 post mating. Serum was separated and stored at -20°C till assayed for total antioxidants. The total antioxidant status was measured using Total Antioxidant Capacity (TAC) Assay Kit (K274-100 BioVision, Inc. Headquarters, 155 South Milpitas Blvd., Milpitas, California 95035).

Statistical analyses

All statistical procedures were performed using the computational software of SAS [12]. Chi-square analysis using the PROC FREQ procedure was used to compare the pregnancy rate among the treatment groups. A t-test was used to analyze the effect of treatments on pregnancy and antioxidants concentration in the studied ewes.

Results

Pregnancy rate detected on days 25 were higher ($P<0.05$) in all the treated groups compared with the control one. However, pregnancy rate and birth weight differed non significantly among G1, G2 and G3 (Table 1). The second group treated with GnRH possessed the longest gestation length, the heavier birth weight and the higher male birth percentage. Different treatment protocols did not improve the incidence of twinning but pregnancy rate was noticeably better than the control. Total antioxidants did not differ significantly between groups in the present study as affected by day of gestation (Table 2). Total antioxidants increased gradually from the day of mating till the 10th day postmating. A significant increase in the level of total antioxidants was observed in all studied groups by the 50th day of gestation.

Treatment	Gestation length	Ewe Body weight	Birth weight	Pregnancy rate%	Male births%	Twining %	Triplets %	litter size
PGF2α	154.1 ± 7.34	36.3 ± 3.42	3.5 ± 0.23	71^{a}	21.42	14.28	----	1.2
GnRH	157.13 ± 8.79	36.12 ± 5.96	3.71 ± 0.41	66^{a}	33.33	25.00	----	1.37
Oxytocin	153.7 ± 6.32	38 ± 9.54	3.29 ± 0.52	71^{a}	13.33	26.66	6.66	1.66
Control	152.44 ± 0.84	38.29 ± 7.67	3.56 ± 0.43	55.54^{b}	15.00	40.00	5.00	1.23

Table 1: Effect of different hormonal regimens on the reproductive performance of Sohagi ewes.

Days after mating	PGF2α	GnRH	Oxytocin	control
0	0.31 ± 0.01^{a}	0.32 ± 0.01^{a}	0.25 ± 0.01^{a}	0.11 ± 0.02^{a}
10	4.23 ± 0.01^{b}	4.04 ± 0.91^{b}	5.58 ± 0.6^{b}	3.22 ± 0.12^{b}
20	4.29 ± 0.45^{b}	5.18 ± 0.37^{b}	5.27 ± 0.59^{b}	4.38 ± 1.09^{b}
30	5.09 ± 0.56^{b}	4.66 ± 0.45^{b}	4.40 ± 0.51^{b}	6.23 ± 0.87^{b}
40	4.99 ± 0.36^{b}	5.22 ± 0.31^{b}	5.36 ± 0.75^{b}	4.91 ± 1.20^{b}
50	7.36 ± 0.42^{c}	7.06 ± 0.93^{c}	6.84 ± 0.74^{c}	8.34 ± 1.65^{c}

Table 2: The Serum concentration of total antioxidant (mmol) in Sohagi ewes treated with different hormonal regimens.

Discussion

With regard to pregnancy rate, the reproductive performance of sheep in the present study improved significantly in treated ewes compared with non treated control ones and notably oxytocin treatment had a positive effect on the litter size. However, birth weight, gestation length and sex ratio as well as total antioxidants were not changed.

The present results of lambing rate in treated groups come close to the results of Beck et al. [13] who found that treatment with GnRH analogue on Day 12 post-mating increased lambing rates and litter size in ewes. In cattle, GnRH improved pregnancy rate by 7-21% [3]. This comes in inconsistency with other studies indicating that pregnancy rate was not affected by GnRH treatment following AI [1,4].

Variability in pregnancy rate among the different studies might be associated with the potency of GnRH on gonadotropin release [14] or the timing of GnRH and mating relative to the onset of estrus. Earlier studies showed that the timing of GnRH injection according to the onset of estrus affected gonadotropin release. Although exogenous GnRH at the onset of estrus increased the pre-ovulatory LH surge [3,4], conception rate increased in one study [3] but not in others [4]. However, the administration of GnRH at the time of AI, approximately 12 hours after the initiation of standing estrus, did not result in a greater surge of LH [15]. In addition, the insufficient LH surge did not have any ovulatory effect [3,15] and did not improve pregnancy. The present results indicated that administration of PGF at the time of AI following spontaneous estrus have a beneficial effect on pregnancy rate. It was suggested that a rapid increase of PGF2α in the ovary may play some important role(s) in the ovulatory process [16,17]. Others reported that prostaglandins of the E series, and particularly PGE, play a crucial role in ovulation by determining the targeting of follicle rupture at the apex, thus allowing release of oocytes to the periovarian space [18]. A prostaglandin analogue, Cloprostenol, administration on the day of estrous of buffalo demonstrated to increase P4 levels on day 11, probably via ET-1 and Ang-II genes inhibition. It has been hypothesized that this phenomenon may be due to specific changes in genes expression, which prevent the intraluteal production of these molecules [6]. If this hypothesis is accepted, the higher pregnancy rates recorded in PG Group could be explained by the reduction of embryo mortality. Moreover, cloprostenol administration in our experiment may have helped ovary contraction and follicle rupture, improving ovulation synchrony. Furthermore, it has been proposed that PGF2a may exert a fertility effect, by causing LH release independent of progesterone withdrawal [19] and that PGF2a administration 30 h before GnRH, elevated the GnRH-induced LH release. It is still unclear if prostaglandin is able to act on LH release by a mechanism different from that induced by GnRH, or if it only enhances GnRH-induced LH release.

In sheep few studies showing the effect of oxytocin on pregnancy rate at the time of AI were published [9,11]. Bekeova et al. [20] indicated that oxytocin, GnRH treatments affected conception rate in post partum ewes through increasing the level of thyroxin, Triiodothyronin, oestradiol 17β and progesterone and suggested that the causes of depression of T4 and T3 levels after parturition in spring might be a lack of gonadotropins. Low concentration of T4 and T3 in certain phases of the post-partum period might be retroactively responsible for the decline in post-partum sexual activity in ewes. However, the study of Yildiz [9] indicated that pregnancy rate increased in lactating dairy cows after oxytocin administration just before AI, which agreed with the present findings. This could be due to changes in uterine contractility and possibly to the acceleration of sperm transport in the reproductive tract of ewes [7,8,21]. Oxytocin possibly exerted its influence by stimulating prostaglandin production [22,23]. In addition to involutory effects upon the uterus [24] prostaglandins may have acted as LH-stimulating [25] and estrogen-stimulating factors [26]. Although there was no significant differences among the experimental groups regarding the level of total antioxidant but it was worthy notable that the level of these elements was gradually increased throughout the early pregnancy period towards the end of the first trimester of the studied ewes. Changes in the antioxidant enzymatic defense could be a part of placentome adaptation to reactive oxygen species-induced oxidative stress at specific early developmental stages of pregnancy. Previous reports showed that the activities of antioxidant enzymes in the sheep corpus luteum (CL) are subject to major changes during early pregnancy, suggesting that the CL of early pregnancy may be rescued from luteolysis through increasing activities of key antioxidant enzymes and inhibition of apoptosis. Maintained levels of antioxidant enzymes in the CL throughout pregnancy may be linked to reactive oxygen species continuously generated in the steroidogenesis activity of luteal cells, and may be involved in the maintenance of luteal steroidogenic activity, cellular integrity and preventive to oxidative stress, improving pregnancy outcomes [27]. Even though the total antioxidant levels were not significantly different, some changes for single antioxidants such as vitamin E, as well as neuroendocrinology-related CART level [28].

Conclusion

In conclusion, the results suggest that the administration of GnRH, oxytocin and PGF at the time of natural mating increased pregnancy rate in subtropical ewes.

References

1. Chenault JR (1990) Effect of fertirelin acetate or buserelin on conception rate at first or second insemination in lactating dairy cows. J. Dairy Sci 73: 633-638.
2. Morgan WF, Lean IJ (1993) Gonadotrophin-releasing hormone treatment in cattle: a meta-analysis of the effects on conception at the time of insemination. Aust Vet J 70: 205-209.
3. Kaim M, Bloch A, Wolfenson D, Braw-Tal R, Rosenberg M, et al. (2003) Effects of GnRH administered to cows at the onset of estrus on timing of ovulation, endocrine responses, and conception. J Dairy Sci 86: 2012-2021.
4. Perry GA, Perry BL (2009) GnRH treatment at artificial insemination in beef cattle fails to increase plasma progesterone concentrations or pregnancy rates. Theriogenology 71: 775-779.
5. Algire JE, Srikandakumar A, Guilbault LA, Downey BR (1992) Preovulatory changes in follicular prostaglandins and their role in ovulation in cattle. Can J Vet Res 56: 67-69.
6. Neglia G, Natale A, Esposito G, Salzillo F, Adinolfi L, et al. (2008) Effect of prostaglandin F2alpha at the time of AI on progesterone levels and pregnancy rate in synchronized Italian Mediterranean buffaloes. Theriogenology 69: 953-960.
7. Sayre BL, Lewis GS (1997) Fertility and ovum fertilization rate after laparoscopic or transcervical intrauterine artificial insemination of oxytocin-treated ewes. Theriogenology 48: 267-275.
8. King ME, Mckelvey WAC, Dingwall WS, Matthews KP, Gebbie FE, et al. (2004) Lambing rates and litter sizes following intrauterine or cervical insemination or frozen/thawed semen with or without oxytocin administration. Theriogenology 62: 1236-1244.
9. Yildiz (2005) Effect of oxytocin on conception rate in cows. J. Firat Uni. Health. Sci 19: 75-78.
10. Cooper MD, Newman SK, Schermerhorn EC, Foote RH (1985) Uterine contractions and fertility following clitoral massage of dairy cattle in estrus. J Dairy Sci 68: 703-708.
11. Hays RL, Van Demark NL, Ormiston EE (1958) Effect of oxytocin and epinephrine on the conception rate of cows. J. Dairy Sci 41: 1376-1379.
12. SAS (2001) Statistical Analysis System: A user's Guide. Version 8.2. Institute Inc.Cary, NC.
13. Beck NFG, Peters AR, Williams SP (1994) The effect of GnRH agonist (buserelin) treatment on day 12 post mating on the reproductive performance of ewes. Anim, Prod. 58: 243-247.
14. Souza AH, Cunha AP, Silva EPB, Gümen A, Ayres H, et al. (2009) Comparison of gonadorelin products in lactating dairy cows: efficacy based on induction of ovulation of an accessory follicle and circulating luteinizing hormone profiles. Theriogenology. 72: 271-279.
15. Lucy MC, Stevenson JS (1986) Gonadotropin-releasing hormone at estrus: luteinizing hormone, estradiol, and progesterone during the periestrual and postinsemination periods in dairy cattle. Biol Reprod 35: 300-311.
16. Iesaka T, Sato T, Igarashi M (1975) Role of prostaglandin F2alpha in ovulation. Endocrinol Jpn 22: 279-285.
17. Armstrong DT (1981) Prostaglandins and follicular functions. J Reprod Fertil 62: 283-291.
18. Gaytán F, Tarradas E, Bellido C, Morales C, Sánchez-Criado JE (2002) Prostaglandin E(1) inhibits abnormal follicle rupture and restores ovulation in indomethacin-treated rats. Biol Reprod 67: 1140-1147.
19. Cruz LC, do Valle ER, Kesler DJ (1997) Effect of prostaglandin F2 alpha- and gonadotropin releasing hormone-induced luteinizing hormone

releases on ovulation and corpus luteum function of beef cows. Anim Reprod Sci 49: 135-142.

20. Bekeova E, Krajnicakova M, Hendrichovsky V, Maracek I (1995) The effects of long-acting oxytocin, GnRH and FSH administration on thyroxin, triiodothyronin, oestradiol 17-fl and progesterone levels as well as conception rates in post-partum ewes. Anim. Reprod. Sci 37: 311-323.
21. Hawk HW (1983) Sperm survival and transport in the female reproductive tract. J Dairy Sci 66: 2645-2660.
22. Kittok RJ, Britt JH (1977) Corpus luteum function in ewes given estradiol during the estrous cycle or early pregnancy. J Anim Sci 45: 336-341.
23. McCracken JA, Schramm W, Okulicz WC (1984) Hormone receptor control of pulsatile secretion of PGF2P from ovine uterus during luteolysis and its abrogation in early pregnancy. Anim Reprod Sci. 7: 31-55.
24. Fredriksson G (1985) Release of PGF(2alpha) during parturition and the postpartum period in the ewe. Theriogenology 24: 331-335.
25. Agmo A (1975) Effects of prostaglandins E-1 and F-2ALPHA on serum luteinizing hormone concentration and on some sexual functions in male rabbits. Prostaglandins 9: 451-457.
26. Dodson KS, Watson J (1980) Stimulatory action of prostaglandin F2 alpha on androgen aromatization in the pig follicle. Eur J Obstet Gynecol Reprod Biol 11: 49-56.
27. Al-Gubory KH, Bolifraud P, Germain G, Nicole A, Ceballos-Picot I (2004) Antioxidant enzymatic defence systems in sheep corpus luteum throughout pregnancy. Reproduction 128: 767-774.
28. Mao J, Ren X, Zhang L, Van Duin DM, Cohen RC, et al. (2012) Insights into hydroxyl measurements and atmospheric oxidation in a California forest. Atmos. Chem. Phys 12: 8009-8020.